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The objective of this proposal is to develop new therapeutic reagents for breast cancer using diabody-based molecules with affinity for HER2/neu for the radioimmunotherapy (RAIT) of breast cancer. The first Technical Objective (T.O.) has focused on the optimization of the production of the selected diabody and the identification of the optimal radionuclide and labeling strategy for diabody-based RAIT. This T.O. also has involved an investigation into the impact on diabody targeting of factors likely to be encountered in a clinical setting. These include the degree of antigen density, the route (i.v. bolus or continuous infusion) and frequency of administration, the presence of disseminated disease, and the effect of antigen expression on normal tissues. Completion of these experiments has set the stage for proceeding to the clinical evaluation of diabody-based targeting of breast cancer in our second Technical Objective. Current experiments demonstrate the feasibility and efficacy of alpha particle based radioimmunotherapy wherein the radionuclide is conjugated to a chelated diabody molecule. The clinical component of this proposal will entail a Phase I radioimmunoimaging and radioimmunoguided surgery trial to elicit information on the dosimetry, specificity and tumor penetration properties of radiolabeled C6.5 diabody, and will assess the RAIT potential of this molecule.

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INTRODUCTION:

The objective of this proposal is to develop new therapeutic reagents for breast cancer. It is our hypothesis that improved diabody-based molecules with affinity for HER2/*neu* can be engineered and will prove to be effective vehicles for the radioimmunotherapy (RAIT) of breast cancer. The first Technical Objective (T.O.) has focused on the optimization of the production of the selected diabody and the identification of the optimal radionuclide and labeling strategy for diabody-based RAIT. This T.O. also has involved an investigation into the impact on diabody targeting and RAIT of a variety of factors likely to be encountered in a clinical setting. These include the degree of antigen density, the route (i.v. bolus or continuous infusion) and frequency of administration, the presence of disseminated disease, and the effect of antigen expression on normal tissues. Completion of these experiments has set the stage for proceeding to the clinical evaluation of diabody-based targeting of breast cancer in our second Technical Objective. The potential value of diabody-based RIT is underscored by current experiments that demonstrate the feasibility and efficacy of alpha particle based radioimmunotherapy wherin the radionuclide is conjugated to a chelated diabody molecule. The clinical component of this proposal will entail a Phase I radioimmunoimaging and radioimmunoguided surgery trial designed to elicit information on the dosimetry, specificity and tumor penetration properties of radiolabeled C6.5 diabody, and will assess the RAIT potential of this molecule. The successful execution of this clinical trial awaits the identification of the support to produce clinical-grade diabody.

BODY:

During the fourth year of this award we have continued to optimize diabody-based RAIT, with particular emphasis placed on understanding the properties of the diabody and identifying the optimal therapeutic radioisotope partner. The accomplishment in this area was the performance of the first preclinical therapy study employing the short-lived radioisotope astatine-211 (^{211}At) conjugated to an engineered antibody-based molecule. This study (described in detail below) demonstrated the efficacy of treating solid tumors with ^{211}At-conjugated diabody molecules. It should be emphasized that these studies are very complex and time consuming due to the difficulty in obtaining sufficient quantities of the radionuclide to permit therapy studies. Numerous experiments are in progress, but the prolonged time required to achieve survival endpoints is reflected by a paucity of current finalized experiments. Only finalized experiments are included here.

It should be noted that our overarching goal has been to obtain additional support to produce GMP lots of diabody for clinical trials. In this regard we applied for DOD support to achieve this goal, but did not obtain the support despite having achieved all the major milestones required to move forward into the clinic. While we remain understandably frustrated by this continuing impediment to translating our preclinical research into human trials, we continue to pursue multiple avenues to produce diabody that can be tested in clinical trials.

KEY RESEARCH ACCOMPLISMENTS:

- We have achieved all our initial specific aims with the exception of conducting a clinical trial of the ^{90}Yttrium-labeled diabody. We remain eager to proceed with this trial, but cannot do so unless we are able to secure support to produce GMP lots of antibody. While striving to achieve this important goal, we have continued to conduct important preclinical studies that point to new directions for diabody-based radioimmunotherapy, as listed below.

- *Conducted preclinical therapy studies evaluating the efficacy of ^{211}At-C6.5 diabody therapy of established tumors in nude mice.* Alpha particles have a short track length (approx. one cell in diameter). However over the course of this track length they deliver exponentially greater energy than that delivered by a beta particle. In the nucleus this high energy leads to unrepairable double stranded breaks in DNA (beta particles cause repairable single strand breaks). As such, a single alpha particle can lead to the death of a tumor cell. In general, alpha particles are emitted from radioisotopes that are too short-lived for pairing with intact antibodies. However, the 7 hour half-life of one particular alpha particle-emitting radioisotope, ^{211}At, is ideally suited for the pharmacokonetics of the C6.5 diabody. *N*-succinimidyl *N*-(4-[^{211}At]astatophenethyl) succinimate (^{211}At-SAPS) was produced by our collaborators, Drs. Brechbiel and Waldmann of the National Cancer Institute. The compound was shipped to our lab where it was conjugated to the C6.5 diabody.

Dose escalation studies were performed with increasing doses of ^{211}AT-SAPS-C6.5 diabody specific for the HER2 tumor-associated antigen. Mice bearing established s.c. human MDA-MB-361.DYT2 breast cancer xenografts were treated with a single dose ranging from 15 to 45 μCi of ^{211}AT-SAPS-C6.5 diabody. In these studies, a clear dose response was observed with the greatest anti-tumor effect associated with the highest treatment dose (Figure 1). In the 45 μCi dose group, three of five treated animals exhibited durable complete responses, with no sign of tumor through the writing of this report (160 days post treatment) (Figure 2). In the dose ranges studied, the maximum tolerated dose has yet to be reached. All of the mice treated with ^{211}AT-SAPS-C6.5 diabody have survived the treatment with minimal observed toxicity (minor transient weight loss). As the tumors that did grow out exhibited a sudden increased rate of growth at about 35 days post treatment, the decision was made to explore the efficacy of using fractionated doses of the ^{211}AT-SAPS-C6.5 diabody at intervals of 30 days. At the

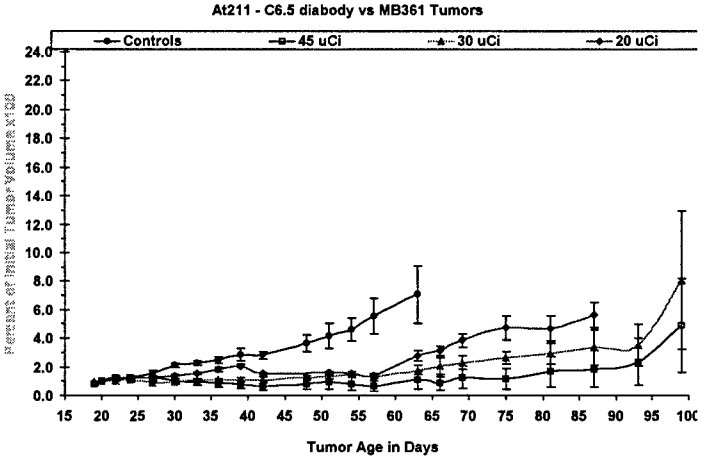

Figure 1. Mice bearing established human MDA-MB-361.DYT2 breast cancer xenografts were treated with a single dose ranging from 15 to 45 μCi of ^{211}AT-SAPS-C6.5 diabody. n = 5-7 per group. Error bars = SEM.

time of this report, five cohorts of mice were treated with a single dose of 25 μCi of ^{211}AT-SAPS-C6.5 diabody. Four cohorts will receive a second dose two weeks from now (day 30 post the first treatment). Finally, the specificity of this therapy is being examined. The same dose range (15 to 45 μCi) that was used in the study described above and in Figure 1 has been employed in an identical study performed using the T84.66 diabody that is specific for CEA, an antigen that is not expressed in the tumor model employed. This study is still underway. The early results indicate that the control ^{211}AT-SAPS-T84.66 diabody does exhibit some anti-tumor effects, however, no complete responses have been observed (data not shown).

REPORTABLE OUTCOMES:

- Manuscripts:

Adams, G.P., Shaller, C.C., Chappel, L. Wu, C., Horak, E.M., Simmons, H.H., Litwin, S., Marks, JD., Weiner, L.M. and Brechbiel, M.W. Delivery of the alpha-emitting radioisotope Bi-213 to tumors via single-chain and diabody molecules. Nucl. Med. Biol., 27:339-346, 2000.

Nielsen, U.B., Adams, G.P., Weiner, L.M. and Marks, J.D.. Targeting of bivalent anti-HER2/*neu* diabody antibody fragments to tumor cells is independent of the intrinsic antibody affinity. Cancer Research, 60:6434-6440, 2000.

Adams, G.P., Shaller, C.C., Dadachova, K., Simmons, H.H., Horak, E.M., Marks, J., Brechbiel, M.W., and Weiner, L.M. Treatment of Established Human Tumor Xenografts in Mice with ^{90}Y-CHX-A" C6.5 Diabody (In preparation).

- Abstracts/Presentations:

Adams, G.P. Tumor targeting and Radioimmunotherapy with single-chain Fv and diabody molecules. Oral presentation at the 17th International Conference on Advances in the Application of Monoclonal Antibodies in Clinical Oncology., June 2000, Samos, Greece.

Adams, G.P., Shaller, C.S., Horak, E.M., Simmons, H.H., Dadachova, K., Chappell, L.L., Wu, C., Marks, J.D., Brechbiel, M.W. and Weiner, L.M. Radioimmunotherapy of established solid tumor xenografts with alpha and beta emitter-conjugated antiHER2/neu single-chain Fv and diabody molecules. Oral presentation at the Eighth Conference on Radioimmunodetection and Radioimmunotherapy of Cancer (Princeton, N.J.) Cancer Biotherapy & Radiopharmaceuticals, 15:402, 2000.

Adams, G.P. Single-chain Fv and Diabody Molecules: Tumor Targeting Properties and Radioimmunotherapy Studies. Presented at the First China International Symposium on Antibody Engineering: Technology and Applications (Tianjin, China) September 2000.

Adams, G.P. Effects of Antibody Structure and Affinity on Tumor Targeting and Penetration. Presented at IBC's 11th Annual International Conference on Antibody Engineering (La Jolla, CA), December 2000.

Adams, G.P. Tumor targeting and Radioimmunotherapy with single-chain Fv and diabody molecules. 18th International Conference on Advances in the Application of Monoclonal Antibodies in Clinical Oncology. June 2001, Vouliagmeni, Greece.

Horak EM, Heitner T, Altomare DA, Garrison JL, Shahied LS, Shaller CC, Tesfaye A, Simmons HH, Alpaugh RK, Greer NB, Testa JR, Marks JD, Weiner LM, Adams GP. Era of Hope 2002 Department of Defense Breast Cancer Research Program Meeting. (Submitted, March, 2002).

CONCLUSIONS:

We continue to optimize C6.5 diabody-based RAIT. While 90Yttrium is an excellent radionuclide partner, ongoing work indicates that 211Astatine offers an improved efficacy and toxicity profile. While we will continue our work to evaluate astatine-211, we feel that yttrium-90 based therapy has demonstrated sufficient efficacy to justify the initiation of a phase I clinical trial. Accordingly continue attempts to secure funding for the production of GMP diabody.

ACRONYMS AND ABREVIATIONS

CD-20	A cell surface protein present on B-cells and B-cell lymphomas.
CHX-A"	A chelating agent capable of binding radiometals to a protein
EGFR	Epidermal Growth Factor receptor
HER2/neu	A protein of the Epidermal Growth Factor receptor family
MTD	Maximum tolerated dose
%ID/g	Percentage of the injected dose localized per gram of tissue
RAID	Radioimmunodetection
RAIT	Radioimmunotherapy
RIGS	Radioimmunoguided surgery
ScFv	Single-chain Fv molecule
SEM	Standard error of the mean

REFERENCES

None

APPENDICES

None